Electron Tomography of Whole Cells

- filling the gap between conventional microscopy and protein crystallography

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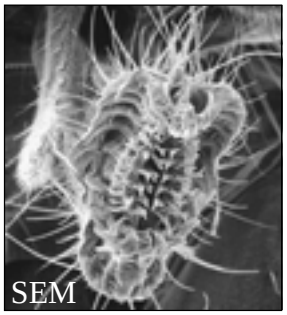
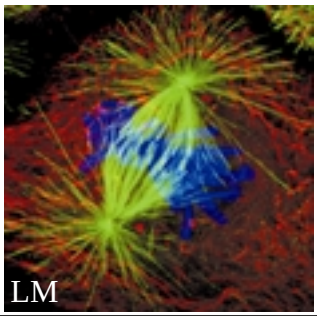
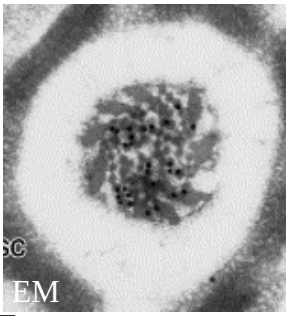
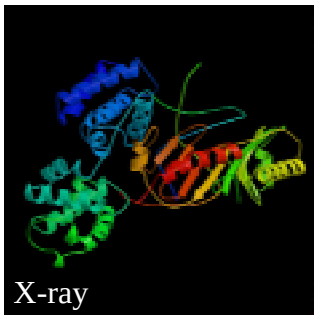
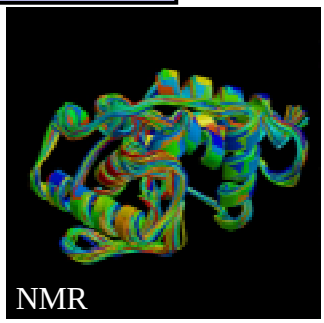






Whole-Cell Tomography





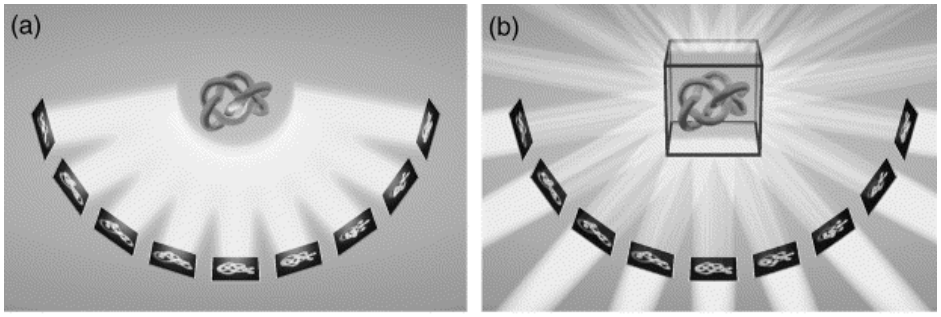
The gap between traditional EM and structural genomics initiatives

- Cellular contexts
- Architecture of many multidomain proteins and complexes
- Conformational variability
- Structures of many membrane proteins
- Many high resolution details

Fundamental challenges in biological EM

- Preserving native structure within microscope
- Obtaining 3D information from projections
- Dose limitations
- Technology well developed for molecular imaging, not so much for tomography

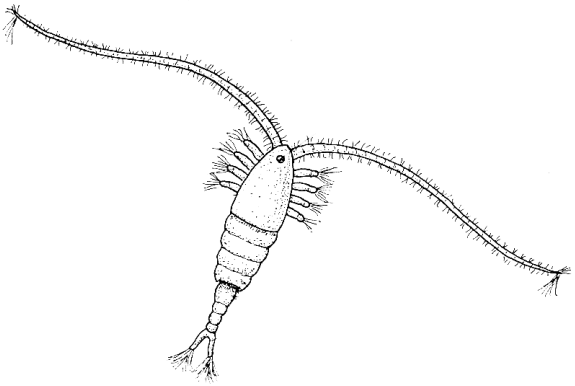
Principles of Tomography



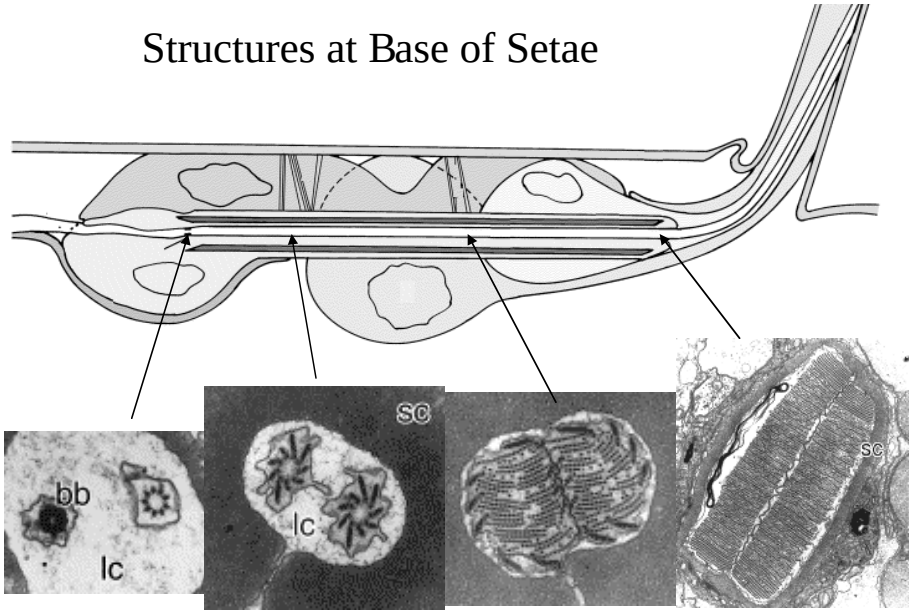
Baumeister et al., *Trends in Cell Biology* **9**:81

A first application - mechanosensory apparatus of copepods

Microtubule-based structure,
visualized by conventional embedding & sectioning

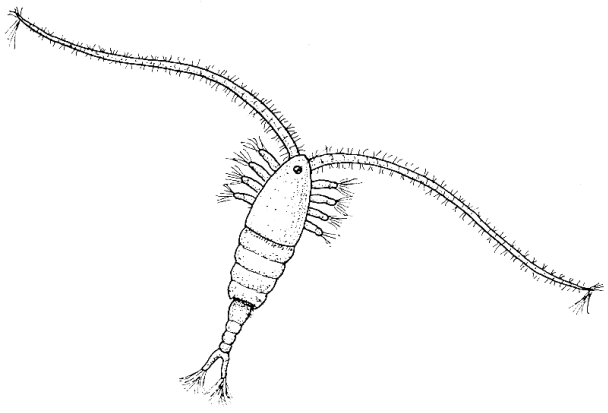


Structures at Base of Setae

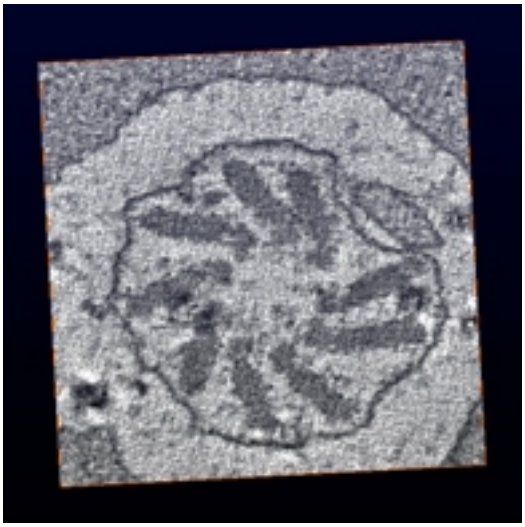


Weatherby and Lenz, *Arthropod Structure and Development*, 29:275

Sectioning through a 3-D reconstruction



Segmenting volume identifies components



“Minimal” cell tomography

- Goal - to map major protein complexes within the cell
- Mycoplasma genitalium, mesoplasma florum
- Smallest free-living organisms known (as small as .2 microns in diameter)
- Only 300-500 genes
- MG - Target of Berkeley high-throughput X-ray crystallography consortium

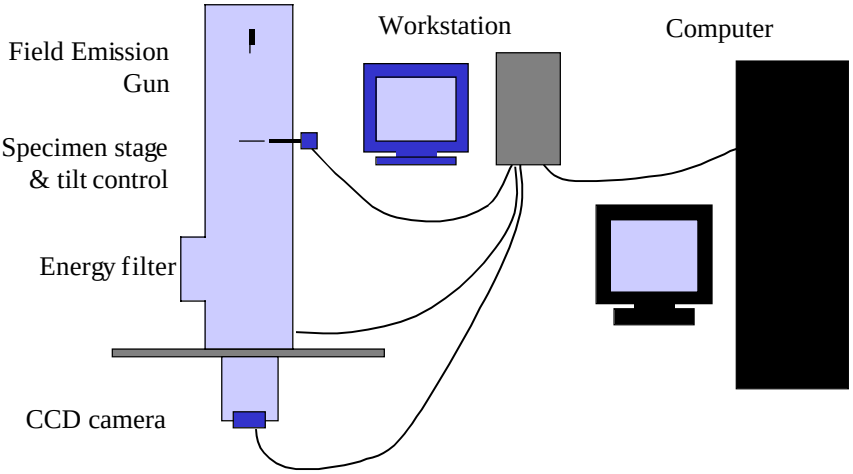
The technology of tomography

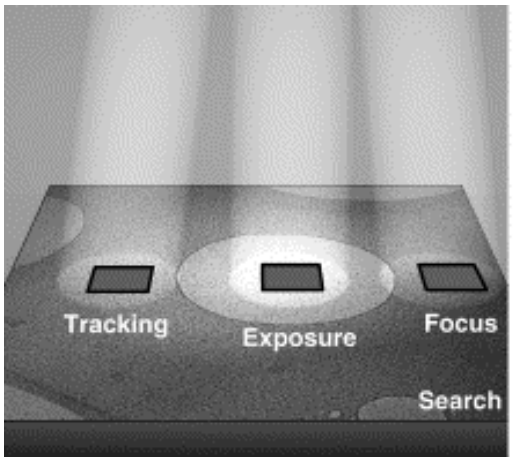
Requirements to meet the goals --

- an electron microscope with:
  - eucentric goniometer
  - 300 kV
  - FEG
  - liquid Helium cooling
  - energy filter
- specimen prepared in ‘native’ state
- computer matching of observed densities

JEOL-3100FFC at LBNL

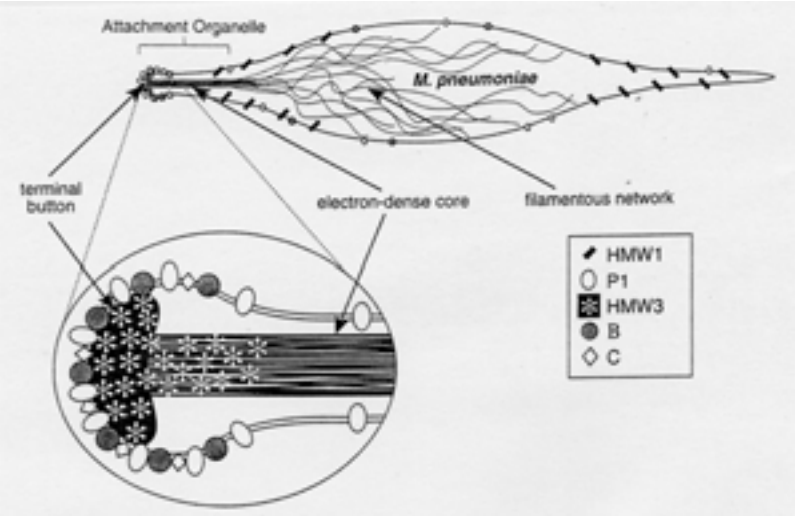
Essentials for Tomography





Baumeister et al., *Trends in Cell Biology* **9**:81

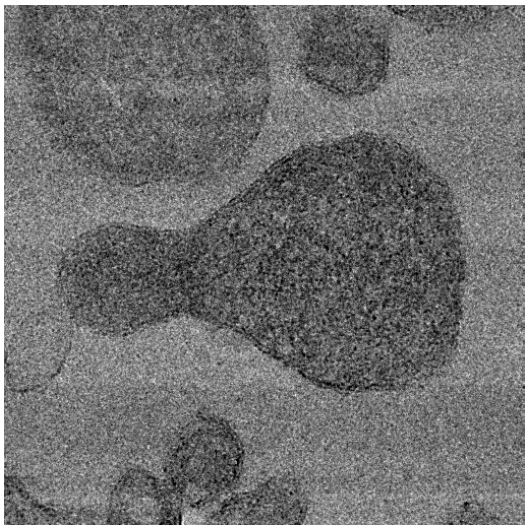
Mycoplasma genitalium



Krause, *Mol. Microbiol.* **20**:247

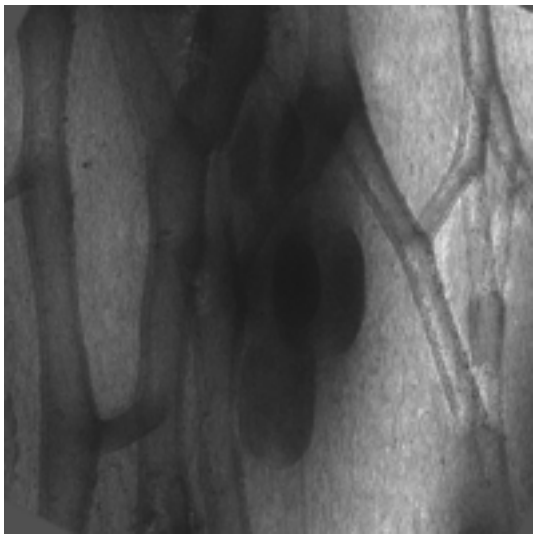
Mycoplasma genitalium

-- visualized by conventional methods



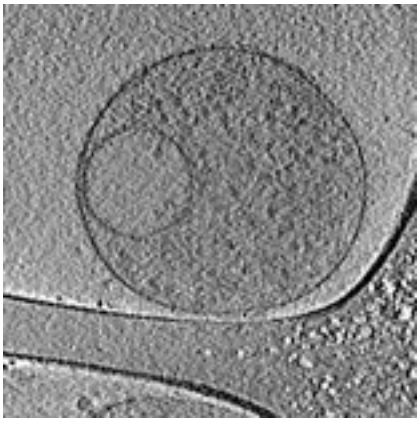
Mesoplasma florum

ice-embedded
using state-of-the-art equipment

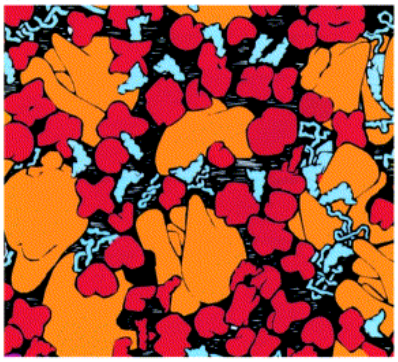


Sectioning through MF 3-D volume

Segmented / Interpreted volume



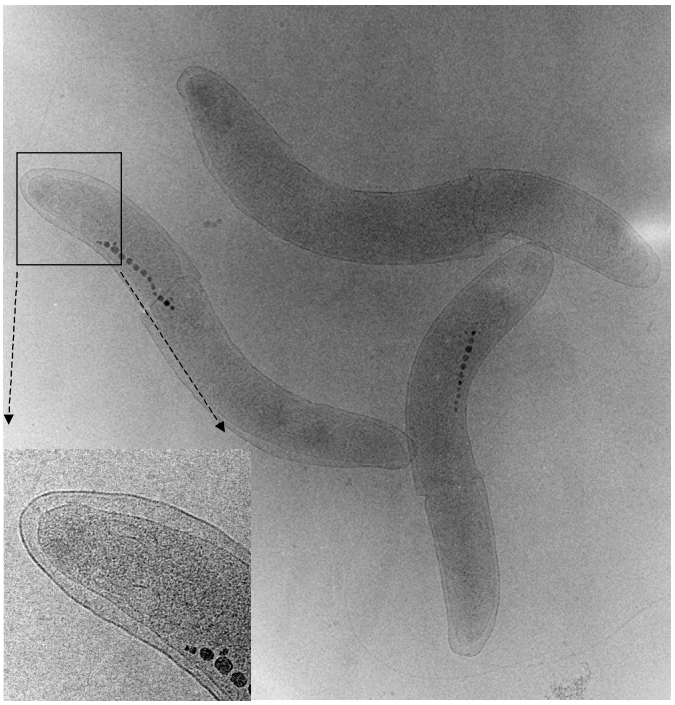
MF internal structure, complexity of prokaryotic cell



Ellis, R.J. (2001)
Current Op. in Struct. Biol. 11:114

Magnetospirillum serpens

- magnetotactic bacteria
- precipitates iron in magnetite particles
- thickness is compatible with electron microscopy
- imaged in frozen-hydrated preparations
- substructures visible even in projection



Conclusions

Electron tomography can still benefit from significant technical development

Already we can see a wealth of internal structure in intact cells prepared in a “native” state.

Eventually should be able to identify locations and interactions of macromolecules